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☐ 1: Cell 1989 Jun 2;57(5):847-57

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Recovery of *Agrobacterium tumefaciens* T-DNA molecules from v plants early after transfer.

Bakkeren G, Koukolikova-Nicola Z, Grimsley N, Hohn B.

Friedrich Miescher-Institut, Basel, Switzerland.

A system for the analysis of independent T-DNA transfer events from *Agrobacterium tumefaciens* plants is described. The complete T-DNA except for the 25 bp border sequences replaced by one genome of a plant virus so that upon transfer to the plant, a vi replicon is produced by circularization. Rescue of virus from such infected plants: analysis of DNA sequences at or close to the ends of T-DNA molecules. A rather right border remnant of three nucleotides was found, whereas the sequences at the left end were more variable. A point deletion in the left 25 bp sequence resulted in less precise processing at the left end. In addition, many rescued T-DNA molecules contained small direct repeats between the joined T-DNA ends; linear T-DNA molecules are transported to the plant.

PMID: 2720788 [PubMed - indexed for MEDLINE]

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☐ 1: Plant Cell 1996 May;8(5):873-86

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Early transcription of *Agrobacterium* T-DNA genes in tobacco and

Narasimhulu SB, Deng XB, Sarria R, Gelvin SB.

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 4 USA.

We developed a sensitive procedure to investigate the kinetics of transcription of *Agrobacterium tumefaciens* transferred (T)-DNA-encoded beta-glucuronidase *gusA* gene soon after infection of plant suspension culture cells. The procedure uses a transcriptase-polymerase chain reaction and enables detection of *gusA* transcripts 18 to 24 hr after cocultivation of the bacteria with either tobacco or maize cells. Expression of *gusA* transcripts depended absolutely on the intact virulence (*vir*) genes *virB*, *virD1/virD2*, and *virD4* within the bacterium. Mutations in *virC* and *virE* resulted in highly attenuated expression of the *gusA* gene. A nonpolar transposon insertion in the C-terminal coding region of *virD2* resulted in only slightly decreased production of *gusA* mRNA, although this insertion resulted in the loss of the nuclear localization signal and the important omega region from VirD2 protein and rendered the bacterium avirulent. However, expression of *gusA* transcripts in tobacco infected by this virus was more transient than in cells infected by a wild-type strain. Infection of tobacco with an *Agrobacterium* strain harboring a mutant *virD2* allele from which the omega region had been deleted resulted in similar transient expression of *gusA* mRNA. These results indicate that the C-terminal nuclear localization signal of the VirD2 protein is not required for nuclear uptake of T-DNA and further suggest that the omega domain of VirC is required for efficient integration of T-DNA into the plant genome. The findings that the initial kinetics of *gusA* gene expression in maize cells are similar to those shown in tobacco cells but that the presence of *gusA* mRNA in maize is highly transient suggest that the block to maize transformation involves T-DNA integration and not T-DNA nuclear targeting.

PMID: 8672885 [PubMed - indexed for MEDLINE]

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